Caenorhabditis elegans: **Stage Specfic Differences in Cuticle Surface Carbohydrates**[1]

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Abstract: Stage-specific differences in wheat germ agglutinin (WGA) binding saccharides were demonstrated between the surfaces of the eggs, L1 larvae, young adults, and old adults of *Caenorhabditis elegans*. The WGA binding was to n-acetylglucosamine groups but not to terminally linked n-acetylneuraminic acids. An age-related decrease in WGA binding occurred in adults, supporting previous findings of a decrease in net negative cuticle surface charge during aging. *Key words:* ^{125}I wheat germ agglutinin, development.

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Stage-specific differences in the chemical composition of the cuticle of *Caenorhabditis elegans* have been demonstrated (3,4). We have examined the surface architecture of the *Caenorhabditis elegans* cuticle, with the ultimate goals of identifying possible cuticular receptors and elucidating changes in molecular makeup which could influence behavior of the larvae or adults during aging.

As an interface between the nematode's external environment and its cellular systems, the cuticle surface is a region of great interest. Recent evidence that cuticular surface saccharides act as receptors which play a role in host finding, and in the identification and attachment of nematophagous fungi to their nematode hosts, may lead to a molecular basis for understanding of recognition phenomena between soil organisms (15). Mannose or glucose residues are localized in the cephalic region of *C. elegans* (9); blockage of the binding (negatively charged) groups on these sugars results in a loss in the ability of the nematode to respond to a strong chemotactic factor (unpublished data). Sialic acids are localized in the head region of *Panagrellus redivivus* (7). Abolition of these binding sites with neuraminidase results in a significant reduction in the ability of the conidia of the parasitic fungus *Meria coniospora* to adhere to the nematode (7) and a reduction in chemoattraction of the nematode to the fungus (B. Nordbring-Hertz and H. B. Jansson, unpublished data). The results of these investigations and others (13) support a hypothesis that the nematode surface contains a number of different highly specific chemical moieties which act as receptors for binding an array of different chemotactic factors.

The objectives of the current study were to examine stage-specific differences in binding of the lectin wheat germ agglutinin (WGA) and to determine if significant attrition of WGA-specific surface sugars occurred during aging of the adult.

MATERIAL AND METHODS

Culturing and age synchrony: C. elegans was maintained axenically in mass culture at 22 C in a medium consisting of 4 g yeast extract, 3 g soy peptone, 10 ml liver extract, and 90 ml water. For each experiment, six 50-ml, 21-day-old cultures were combined and the eggs, L1 larvae, young adults (5–7 days old), and old adults (17–21 days old) segregated into age-synchronous groups.

Age synchrony was attained in several ways. For eggs, egg clusters from mass culture were washed three times with distilled water to remove young larvae and then placed in tris buffer (pH 7.2) (12).

Larvae and adult nematodes were segregated from mass culture by a modification of the method of Bollinger and Willett (2) for synchronizing age groups of *P. redivivus* from xenic culture.

Live nematodes were separated from the dead by placing the nematodes on a Baermann funnel for 3 hours and collecting the nematodes which passed through the filter. The nematodes were washed twice in 0.3% NaCl and concentrated by centrifugation prior to segregation on a sucrose gradient column. The column consisted of 250 ml

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of 3–10% sucrose in a linear gradient in a 2.5 × 45-mm buret. The blended solution was allowed to equilibrate for 1 hour. The concentrated nematodes were carefully pipetted onto the top of the gradient and allowed to segregate for 40 min. At the end of this period, the upper 30 ml of the gradient consistently contained 100% L1 larvae, hence synchrony of this group was attained by removing this fraction by pipette, washing three times in Tris buffer (pH 7.2), and concentrating the nematodes by centrifugation.

The remaining 220 ml were drawn from the buret in 20-ml fractions. The first two fractions contained a pure population of old nematodes, judged to be 17–21 days old on the basis of having body dimension and appearance similar to nematodes of like age which had been maintained in separate vials throughout their life spans.

The major fractions containing young adults (5–7 days old) were fractions 6 and 7. Synchrony of this age group was not complete, with variations of from 5 to 30% of the nematodes being older or younger in different runs. When the percentage of variants exceeded 10, these fractions were discarded. However, with care and repetition, sufficient replicates which consisted mostly of young adults were obtained. These nematodes were also washed three times with Tris buffer (pH 7.2) before further processing.

Preparation of ^{125}I WGA: WGA was purified by affinity chromatography, labelled with ^{125}I by the chloramine-T reaction (14), and repurified by affinity chromatography following the methods of Kahane and Tully (8). The concentration of WGA was 15 μg protein/ml.

Binding of lectins: WGA binds specifically to n-acetylneuraminic acid, n-acetyl-D-galactosamine (not strongly), and n-acetyl-D-glucosamine and its β-(1–4) linked oligosaccharides (1,10,11). The binding experiments proceeded as follows: ^{125}I-WGA (specific activity 12 mc/mg) in 0.005 M Tris-hydrochloride (pH 7.2) was added to the nematode suspension to make up 1 ml of the same buffer and incubated at 20–22 C for 30 min. The nematodes were centrifuged at 9,000 g, a sample aliquot taken of the supernatant for radioassay, and the remaining supernatant discarded. One milliliter of buffer was added and the pelleted nematodes resuspended by Vortex mixer. This procedure was repeated through four buffer washes, at which time radioassay indicated that essentially all unbound WGA had been removed.

Specificity of binding was estimated (16) by competitive displacement of the bound WGA by its specific sugar n-acetylglucosamine (2.2 g/100 ml in Tris-HCl, pH 7.2). Nonspecific binding was estimated by exposure to α-methyl-D-mannoside (1.94 g/100 ml Tris-HCl, pH 7.2).

Following the buffer washes, the nematodes were sequentially incubated for 20 min each in two washes of α-methyl-D-mannoside, 20 min each in two washes of n-acetyl-D-glucosamine, and then subjected to a final buffer wash. At each step, an aliquot was taken for radioassay, the remaining supernatant discarded, and the nematodes pellet resuspended by Vortex mixing. The amount of lectin remaining in the sample at the completion of the test was also determined (see Table 1).

In each experiment, separate samples containing eggs, L1, young adults, or old adults were run in tandem. Each experiment was replicated five times.

Two trials were run to determine if the distribution of WGA-specific sugars could be visualized following treatment with a FITC-WGA conjugate (Miles Biochemicals Elkhart, Indiana). The binding protocol was the same as followed with ^{125}I-la-

Table 1. WGA binding to the surfaces of several developmental stages of *Caenorhabditis elegans**

Nematode stage	Binding (%)†	Displacement (%)‡
Egg	9	55
Larvae (L1)	7	69
Young adult	3	53
Old adult	5	54

*Each figure represents an average of five trials.

†Amount of lectin specifically bound to the nematode. The figures derive from radioassays of sample aliquots drawn from the iodinated lectin initially added to the nematode suspension and the supernatant of samples exposed to the specific sugar.

‡Amount of specifically bound lectin competitively displaced by the specific sugar as compared to total radioactivity bound to nematodes following four buffer washes.

belled WGA, to the point of a specific displacement by a competitive sugar. At this stage, the nematodes were placed directly in buffer and viewed under a fluorescence microscope.

Normalization of data: The data were normalized so that the comparative densities of the WGA-specific sugars on the outer surfaces of the four developmental stages of *C. elegans* could be evaluated. The surface area for each stage was calculated as follows: the egg was considered as a cylinder + 2 hemispheres (hence $2\pi rh + 4\pi r^2$) and the vermiform stages of the nematode as a cylinder (with the minor correction for the conical region from the anus to the tail being disregarded, hence $2\pi rh$). The appropriate measurements were taken of 20 specimens in each group and the surface area calculated (Table 2).

Measurement of numbers of specimens per sample was based on counts from 1/10 of a sample. Corrections were then made for the total surface area in each combination from each test. Initially the samples were adjusted so that each had an equal volume of biological material (Table 2).

RESULTS

WGA binding to the outer cuticular surfaces of the several developmental stages tested varied from 3 to 9% (Table 1). Displacement of specifically bound WGA was consistent between stages, with the exception of the L1 larvae where displacement was higher (Table 1). The displacement data support the finding (Table 2) that L1 larvae contain fewer cuticular WGA-specific binding sites. Displacement (Table 1) is used only to compare the WGA competitively displaced between each nematode stage tested and does not represent all specifically bound WGA. In a typical test, radioassays were as follows: last buffer wash—200 cpm (essentially devoid of unbound lectin); nonspecific displacement—5,100 cpm; specific displacement—22,200 cpm; and radioactivity remaining in the sample at the completion of the test—13,000 cpm. The latter figure represents mostly specifically bound WGA not displaced by the n-acetyl-D-glucosamine. The calculations of radioactivity per unit surface area are based on radioassays of the supernatant containing iodinated lectin competitively displaced by the specific sugar.

The calculations of radioactivity per unit surface area (Table 2) demonstrated the density of WGA binding saccharides on the exposed cuticle surface of *C. elegans*. The density of these saccharides was greatest on young adults and showed a significant decrease ($P = 0.01$) in old adults (Table 2). These results support the previous report of a 21.8% decrement in total negative surface charge in adults during aging of the sibling species *C. briggsae* (5). Eggs showed a lower density of WGA binding sites, and the L1 larvae the lowest density. Differences ($P = 0.01$) in density of WGA binding sites occurred between each of the four developmental stages examined.

Following exposure to FITC-WGA, no detectable fluorescent labelling above background was observed.

Table 2. Relative densities of wheat germ agglutinin binding sites on the surface of several developmental stages of *Caenorhabditis elegans*.*

Nematode stage	Number/sample	Surface area/ sample (μm^2)	cpm/unit surface area (μm^2)	Relative density of binding sites†
Egg	1.7×10^6	8.0×10^9	1.5×10^{-6}	16.6
Larvae (L1)	2.9×10^6	2.5×10^{10}	2.5×10^{-7}	1.0
Young adult	2.6×10^4	2.1×10^9	5×10^{-6}	50.0
Old adult	9.9×10^3	1.7×10^9	6×10^{-6}	41.2

*Data adjusted for total surface area of each developmental stage in a sample.

†Since the LI larvae showed the lowest binding density, WGA binding to this stage was used as a base for comparison. Difference in relative density of binding sites was highly significant ($P = 0.01$) between each of the four stages tested.

DISCUSSION

The inability to detect WGA-specific carbohydrates in the FITC-WGA tests indicate the much higher sensitivity of the ^{125}I labelling method.

WGA binding to the eggs of the plant parasitic nematode *Meloidogyne javanica* previously was reported (12). The current study is the second finding of WGA-specific sugars on the surface of nematode eggs.

The proportional reduction of WGA-specific carbohydrates to total surface carbohydrates, as measured by net negative surface charge (5), suggests that there may be a continuous age-related attrition in all specific binding sites on the glycocalyx during the life of the adult. This hypothesis remains to be tested.

Of the several reported WGA-specific sugars, terminally linked n-acetylneuraminic acids can be eliminated, for in previous tests on *C. briggsae* (6) and *C. elegans* (S. Himmelhoch and B. Zuckerman, unpublished results), the absence of exposed sialic acids was proven by the lack of reduction in labelling by cationized ferritin following exposure to neuraminidase. Of four plant parasitic nematodes studied (13), all showed the presence of sialo residues in the cuticle, but in only one species were the residues terminally situated and therefore available for digestion by neuraminidase. The present studies do not discriminate between the possibilities of specific WGA binding to n-acetylglucosamine and n-acetylgalactosamine, although it is known that the WGA lectin binds avidly to n-acetylglucosamine and less strongly to n-acetylgalactosamine. For this reason, we conclude that the WGA binding to *C. elegans* most probably is to n-acetylglucosamine residues.

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